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(54) Title: METABOTROPIC GLUTAMATE RECEPTORS AND METHODS OF USE THEREFOR

Human GRMX-Ia protein sequence

MVLLLILSVLLLKEDVRGSAQSSERRVVAHMLGDIIIGALFSVHHQPTVDEVHERKCGAV
REQYGIQRVEAMLHTLERINSDPTLLPNITLGCEIRDSCWHSAVALEQSIEFIRDSLISS
EEEEGLVCSVDGSSSSFRSKKPIVGVIGPGSSSVAIQVQNLLQLFNIPQIAYSATIMDLS
DKTLFKYFMRVVPSDAQQARSMVDIVKRYNWTYVSAVHTEEQANCEEDWLQVEQKLIYYK

(57) Abstract: Compounds and methods for modulating glutamate binding are provided. Compounds include human metabotropic glutamate receptor polypeptides, and variants thereof that binds glutamate, or one or more agents that affects the ability of such a polypeptide to bind glutamate. The compounds may be used, for example, in the prevention and treatment of a variety of conditions associated with undesirable glutamate levels, including certain neurological disorders.

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METABOTROPIC GLUTAMATE RECEPTORS AND METHODS OF USE THEREFOR

TECHNICAL FIELD

The present invention relates generally to compositions and methods for the treatment of conditions associated with undesirable levels of glutamate. The invention is more particularly related to metabotropic glutamate receptors and variants thereof that bind glutamate, as well as agents that bind to such receptors. Such receptors and agents may be used, for example, for the therapy of neurological disorders.

BACKGROUND OF THE INVENTION

Glutamate is a major excitatory neurotransmitter in the brain that is involved in modulating synaptic transmission, ion channel activity and synaptic plasticity. The disregulation of glutamate and/or its receptors can contribute to the neurodegeneration seen in diseases like Alzheimer's and Parkinson's as well as stroke. Glutamate and its receptors may also contribute to diseases like depression, schizophrenia, anxiety and pain.

Schizophrenia is a severely debilitating and common disease characterized by various types of disturbed behaviors, thoughts and feelings. Most current therapies are based on antipsychotic medications that function as dopamine D2 antagonists. Such therapies have produced benefit for some patients, but do not fully ameliorate the negative symptoms of schizophrenia.

There is now growing evidence that a reduction in glutamate levels may be, in part, responsible for schizophrenia. The "glutamate hypothesis" was originally developed from the observation that phenycyclidine, an NMDA receptor antagonist, induces a psychotic state that closely resembles schizophrenia in normal individuals. Glycine, which potentiates NMDA receptor activity, has been used as an adjunct to conventional antipsychotics, with a resulting improvement in negative symptoms. Other agents that enhance glutamate levels have the potential to provide further improvement in therapies for schizophrenia.

Accordingly, there is a need in the art for more effective therapies for schizophrenia. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides metabotropic glutamate receptor sequences, and methods employing such sequences. Within certain aspects, isolated polynucleotides that encode a metabotropic glutamate receptor polypeptide are provided. Metabotropic glutamate receptor polypeptides include polypeptides that comprise at least 50 consecutive amino acids of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12, as well as variants thereof that differ in one or more substitutions, deletions, additions and/or insertions at no more than 10% of amino acid residues, such that the immunogenicity or glutamate-binding activity of the variant is not substantially diminished relative to the metabotropic glutamate receptor protein. Metabotropic glutamate receptor polynucleotides may, within certain embodiments, comprise at least 60 consecutive nucleotides of a sequence recited in any one of SEQ ID NOs:1, 3, 5, 6, 7, 9 or 11.

The present invention further provides isolated antisense polynucleotides complementary to at least 20 consecutive nucleotides present within a metabotropic glutamate receptor polynucleotide.

Within related aspects, the present invention provides recombinant expression vectors comprising a metabotropic glutamate receptor polynucleotide or antisense polynucleotide, as well as host cells transformed or transfected with such an expression vector.

Within other aspects, metabotropic glutamate receptors are provided. Such polypeptides may comprise at least 50 consecutive amino acids of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12 or a variant thereof, as described above.

Within other aspects, metabotropic glutamate receptor polypeptides as described above are provided. Such polypeptides may comprise at least 50 consecutive amino acids of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12, or a variant thereof as described above.

Within further aspects, methods are provided for preparing a metabotropic glutamate receptor polypeptide, comprising the steps of: (a) culturing a host cell transformed or transfected with an expression vector comprising a polynucleotide that encodes a

metabotropic glutamate receptor polypeptide as described above; wherein the step of culturing is performed under conditions promoting expression of the polynucleotide; and (b) recovering a metabotropic glutamate receptor polypeptide.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) a metabotropic glutamate receptor or metabotropic glutamate receptor polypeptide as described above; and (b) a physiologically acceptable carrier.

Vaccines are also provided, comprising: (a) a metabotropic glutamate receptor or metabotropic glutamate receptor polypeptide as described above; and (b) a non-specific immune response enhancer.

Within further aspects, the present invention provides isolated antibodies, or antigen-binding fragments thereof, that specifically bind to a metabotropic glutamate receptor polypeptide as described above.

The present invention further provides methods for screening for agents that modulate metabotropic glutamate receptor expression or activity. Within certain such aspects, methods are provided for screening for an agent that modulates metabotropic glutamate receptor expression in a cell, comprising: (a) contacting a candidate modulator with a cell expressing a metabotropic glutamate receptor polypeptide as described above; and (b) subsequently evaluating the effect of the candidate modulator on expression of a metabotropic glutamate receptor mRNA or polypeptide, and therefrom identifying an agent that modulates metabotropic glutamate receptor protein expression in the cell. Similar screens may be performed using a cell comprising a metabotropic glutamate receptor gene promoter operably linked to a reporter gene, and evaluating the effect of a candidate modulator on expression of the reporter gene.

Within further such aspects, methods are provided for screening for an agent that modulates a metabotropic glutamate receptor activity, comprising: (a) contacting a candidate modulator with a metabotropic glutamate receptor polypeptide as described above, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and (b) subsequently evaluating the effect of the candidate modulator on the ability of the metabotropic glutamate

receptor to bind glutamate, and therefrom identifying an agent that modulates an activity of a metabotropic glutamate receptor.

Metabotropic glutamate receptor polynucleotides, polypeptides and modulating agents may be used for a variety of therapeutic applications. Within certain aspects, methods are provided herein for treating a condition associated with undesirable level of glutamate. Such conditions include neurological disorders, such as schizophrenia. Methods for treating such a condition in a patient generally comprise administering to a patient an agent that decreases the ability of a metabotropic glutamate receptor polypeptide to bind glutamate or inhibits expression of a metabotropic glutamate receptor. The agent may be present within a pharmaceutical composition as described above, with or without additional therapeutic agents.

Methods are further provided for modulating metabotropic glutamate receptor expression and/or activity in a cell, comprising contacting a cell expressing an a metabotropic glutamate receptor polypeptide with an effective amount of an agent that modulates a metabotropic glutamate receptor glutamate expression or binding activity; and thereby modulating a metabotropic glutamate receptor expression and/or activity in the cell.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1a (SEQ ID NO:1).

Figure 2 is an amino acid sequence of the representative human metabotropic glutamate receptor GRMX-1a (SEQ ID NO:2).

Figure 3 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1b (SEQ ID NO:3).

Figure 4 is an amino acid sequence of the representative human metabotropic glutamate receptors GRMX-1b, GRMX-1c and GRMX-1d (SEQ ID NO:4).

Figure 5 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1c (SEQ ID NO:5).

Figure 6 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1d (SEQ ID NO:6).

Figure 7 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1e (SEQ ID NO:7).

Figure 8 is an amino acid sequence of the representative human metabotropic glutamate receptor GRMX-1e (SEQ ID NO:8).

Figure 9 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1f (SEQ ID NO:9).

Figure 10 is an amino acid sequence of the representative human metabotropic glutamate receptor GRMX-1f (SEQ ID NO:10).

Figure 11 presents the sequence of a polynucleotide encoding the representative human metabotropic glutamate receptor GRMX-1g (SEQ ID NO:11).

Figure 12 is an amino acid sequence of the representative human metabotropic glutamate receptor GRMX-1g (SEQ ID NO:12).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to polypeptides comprising a human metabotropic glutamate receptor, or a portion or variant thereof. The present invention further provides metabotropic glutamate receptor polynucleotides encoding such polypeptides (or complementary to such coding polynucleotides) and agents that modulate an activity of such polypeptides, such as the ability to bind glutamate. Metabotropic glutamate receptor polypeptides, polynucleotides and/or modulating agents may generally be used for treating conditions associated with undesirable levels of glutamate.

METABOTROPIC GLUTAMATE RECEPTOR POLYNUCLEOTIDES

Any polynucleotide that encodes a metabotropic glutamate receptor polypeptide as described herein is encompassed by the present invention. Such polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be

DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Metabotropic glutamate receptor polynucleotides may comprise at least 30, 60, 100 or 150 consecutive nucleotides of a native metabotropic glutamate receptor sequence (*i.e.*, a metabotropic glutamate receptor gene that can be found in an organism that is not genetically modified), or may comprise a variant of such a sequence. Native metabotropic glutamate receptor sequences encompassed by the present invention include DNA and RNA molecules that comprise a sequence provided herein, as well as homologues thereof from other species and other native metabotropic glutamate receptor sequences that may be identified based on homology to the sequences provided herein. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the ability of the encoded polypeptide to bind glutamate and/or the immunogenicity of the polypeptide is not diminished, relative to a native metabotropic glutamate receptor protein. The effect on glutamate binding activity may generally be assessed using any standard binding assay, and as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or additions at no more than 30%, preferably at no more than 20% and more preferably at no more than 10%, of the nucleotide positions. Certain variants are substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a metabotropic glutamate receptor polypeptide (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to

the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes. Antisense oligonucleotides may be synthesized directly, or cDNA constructs that can be transcribed into antisense RNA may be introduced into cells or tissues to facilitate the production of antisense RNA. Antisense oligonucleotides are preferably at least 20 nucleotides in length, preferably at least 30 nucleotides in length. A portion of a coding sequence or a complementary sequence may also be designed as a probe or primer to detect gene expression. Probes may be labeled by a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers are preferably 22-30 nucleotides in length.

Metabotropic glutamate receptor polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be amplified from cDNA prepared from brain tissue or testis, both of which express metabotropic glutamate receptors. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized. An amplified portion may then be used to isolate a full length gene from a human genomic DNA library or from a suitable cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR fragments. Metabotropic glutamate receptor polynucleotides may also be prepared by synthesizing oligonucleotide components (which may be derived from a sequence provided herein), and ligating components together to generate the complete polynucleotide. One other approach is to screen a library with a synthesized oligonucleotide that hybridizes to a metabotropic glutamate receptor gene. Libraries may generally be prepared and screened using methods well known to those of ordinary skill in the art, such as

those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. One suitable library is a brain cDNA library. Other libraries that may be employed will be apparent to those of ordinary skill in the art.

As noted above, polynucleotides comprising portions and other variants of native metabotropic glutamate receptor sequences are within the scope of the present invention. Such polynucleotides may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding a metabotropic glutamate receptor polypeptide, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Variants may also be generated by mutagenesis or enzymatic digestion of native sequences. Certain polynucleotides may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a polynucleotide may be administered to a patient such that the encoded polypeptide is generated *in vivo*.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (*e.g.*, avian pox virus). Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

METABOTROPIC GLUTAMATE RECEPTORS

As used herein, the term "metabotropic glutamate receptor polypeptide" encompasses amino acid chains of any length. For example, a metabotropic glutamate receptor polypeptide may comprise a full length endogenous (*i.e.*, native) metabotropic glutamate receptor protein. Such a polypeptide may consist entirely of a native sequence, or may contain additional heterologous sequences. Native metabotropic glutamate receptor sequences may generally be identified based on sequence homology to known metabotropic glutamate receptor protein sequences, as described herein. A "metabotropic glutamate receptor protein" may consist entirely of a metabotropic glutamate receptor polypeptide, or may be a multimer comprising such a polypeptide. For example, a metabotropic glutamate receptor protein may be a homodimer, comprising two identical polypeptides, or a heterodimer, comprising two different polypeptides. Within certain embodiments, a

metabotropic glutamate receptor polypeptide comprises at least 50 consecutive amino acids of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12.

A metabotropic glutamate receptor polypeptide may comprise a portion of a native metabotropic glutamate receptor polypeptide. Such a portion is preferably at least 20 consecutive amino acid residues in length, more preferably at least 50 consecutive amino acid residues in length. Within certain embodiments, the portion binds glutamate with an affinity that is not substantially diminished relative to the full length metabotropic glutamate receptor polypeptide. In other words, the ability to bind glutamate may be enhanced, unchanged or diminished by less than 10% relative to the full length polypeptide.

Alternatively, a metabotropic glutamate receptor polypeptide may comprise a variant of a native metabotropic glutamate receptor polypeptide or portion thereof. A "variant" is a polypeptide that differs in sequence from a native protein only in substitutions, deletions, insertions and/or additions. Within certain embodiments, substitutions are made (if at all) at no more than 30%, preferably at no more than 20% and more preferably at no more than 10% of residues within a native metabotropic glutamate receptor polypeptide, as described above. Additional amino acid sequences (such as, for example, linkers, tags and/or ligands), may be located within the polypeptide or, preferably, at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification, detection or cellular uptake of the polypeptide.

Substitutions are preferably conservative. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity on polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu,

asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

As noted above, certain variants retain an activity of a native metabotropic glutamate receptor polypeptide. For example, a variant may retain the ability to bind glutamate. Such a variant may bind glutamate with an affinity that is not substantially diminished relative to the native protein. In other words, the affinity for glutamate of the variant may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein.

Also encompassed by the present invention are splice variants of a metabotropic glutamate receptor polypeptide. Such variants may have one or more domains deleted or replaced by a domain providing a different function. Such splice variants may be identified using amplification or hybridization techniques described herein.

In general, metabotropic glutamate receptor polypeptides may be prepared using any of a variety of techniques that are well known in the art. For example, polypeptides of the present invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are bacteria, yeast, insect or mammalian cells. The recombinant DNA may be cloned into any expression vector suitable for use within the host cell and transfected into the host cell using techniques well known to those of ordinary skill in the art. An expression vector generally contains a promoter sequence that is active in the host cell. A tissue specific promoter may also be used, as long as it is activated in the target cell. Preferred promoters express the polypeptide at high levels.

Optionally, the construct may contain an enhancer, a transcription terminator, a poly(A) signal sequence, a bacterial or mammalian origin of replication and/or a selectable marker, all of which are well known in the art. Enhancer sequences may be included as part of the promoter region used or separately. Transcription terminators are sequences that stop RNA polymerase-mediated transcription. The poly(A) signal may be contained within the termination sequence or incorporated separately. A selectable marker includes any gene that confers a phenotype on the host cell that allows transformed cells to be identified. Such markers may confer a growth advantage under specified conditions. Suitable selectable markers for bacteria are well known and include resistance genes for ampicillin, kanamycin

and tetracycline. Suitable selectable markers for mammalian cells include hygromycin, neomycin, genes that complement a deficiency in the host (*e.g.*, thymidine kinase and TK$^-$ cells) and others well known in the art.

Metabotropic glutamate receptor polypeptides may be expressed in transfected cells by culturing the cell under conditions promoting expression of the transfected polynucleotide. Appropriate conditions will depend on the specific host cell and expression vector employed, and will be readily apparent to those of ordinary skill in the art. For commercially available expression vectors, the polypeptide may generally be expressed according to the manufacturer's instructions. Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and/or affinity chromatography.

Such techniques may be used to prepare native polypeptides or variants thereof. For example, variants of a native polypeptide may generally be prepared from polynucleotide sequences modified via standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides. Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from

some or all of the coexisting materials in the natural system. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

EVALUATION OF GLUTAMATE-BINDING ACTIVITY

As noted above, native metabotropic glutamate receptor polypeptides and certain variants thereof possess the ability to bind glutamate. Such binding activity may generally be evaluated using any standard binding assay. In general, a variant of a metabotropic glutamate receptor polypeptide is said to retain glutamate-binding activity if it binds glutamate with an affinity that is not substantially diminished relative to the affinity of the native metabotropic glutamate receptor polypeptide. In other words, such affinity may be enhanced, unchanged or diminished by less than 10%, relative to the affinity of the native polypeptide.

One assay for detecting glutamate binding is a competition binding assay, which can be performed using radiolabeled ligand specific for the metabotropic glutamate receptor in question. Since the majority of these receptors are retained within the endoplasmic reticulum, binding can be measured using membrane preparations from transfected HEK-293 cells. As an alternative, the receptor genes can be expressed in baculovirus. In addition one can generate truncated constructs that encode only the extracellular ligand binding domain of the metabotropic glutamate receptor. To accomplish this, a histidine tag can be placed at the carboxy terminal end of the protein, which will terminate just prior to the first membrane spanning domain. This recombinant protein can then be captured using nickel coated sepharose beads and the protein used in a binding assay as described above.

In a second method, cDNAs for metabotropic glutamate receptors can be converted into cRNA and injected into Xenopus oocytes. Activation of type I metabotropic glutamate receptors (such as mGluR5) with glutamate stimulates phospholipase C which in turn leads to the activation of an oscillatory Cl^- current via the release of intracellular Ca^{2+}. This current can be measured using patch-clamp technology.

In a third method, recombinant cDNAs enoding a metabotropic glutamate receptor polypeptide can be co-transfected into HEK-293 cells. The cells are then loaded with the calcium sensitive dye Fura-2 and stimulated with glutamate. The release of Ca^{2+} from intracellular stores can be measured using real-time Ca^{2+} imaging.

METABOTROPIC GLUTAMATE RECEPTOR MODULATING AGENTS

The present invention further provides agents capable of modulating metabotropic glutamate receptor activity. Such agents may function by modulating metabotropic glutamate receptor protein transcription or translation, by stabilizing or destabilizing a metabotropic glutamate receptor protein, or by directly inhibiting or enhancing an activity, such as glutamate binding activity, of a metabotropic glutamate receptor protein. Preferably, a modulating agent has a minimum of side effects and is non-toxic. For some applications, agents that can penetrate cells or that are targeted to interstitial spaces are preferred.

Modulating agents include substances that selectively bind to a metabotropic glutamate receptor protein. Such substances include antibodies and antigen-binding fragments thereof (*e.g.*, $F(ab)_2$, Fab, Fv, V_H or V_K fragments), as well as single chain antibodies, multimeric monospecific antibodies or fragments thereof and bi- or multi-specific antibodies and fragments thereof. Antibodies that bind to a metabotropic glutamate receptor protein may be polyclonal or monoclonal, and are specific for a metabotropic glutamate receptor polypeptide (*i.e.*, bind to such a polypeptide detectable within any appropriate binding assay, and do not bind to an unrelated protein in a similar assay under the same conditions). Preferred antibodies are those antibodies that function as modulating agents to inhibit or block a metabotropic glutamate receptor protein activity *in vivo*. Antibodies may also be employed within assays for detecting the level of metabotropic glutamate receptor protein within a sample.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g.*, mice, rats, rabbits, sheep and

goats), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Once a cell line, such as a hybridoma, expressing an antibody that specifically binds to a metabotropic glutamate receptor protein has been obtained, other chimeric antibodies and fragments thereof as described herein may be prepared. Using well known techniques, a cDNA molecule encoding the antibody may be identified.

Other modulating agents include peptides, and nonpeptide mimetics thereof, that specifically interact with one or more regions of a metabotropic glutamate receptor

protein. Such agents may generally be identified using any well known binding assay, such as a representative assay provided herein. For example, such modulating agents may be isolated using well known techniques to screen substances from a variety of sources, such as plants, fungi or libraries of chemicals, small molecules or random peptides.

Other modulating agents may function by inhibiting or enhancing transcription or translation of a metabotropic glutamate receptor gene. For example, modulating agents may include antisense polynucleotides (DNA or RNA), which inhibit the transcription of a native metabotropic glutamate receptor protein. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA. Antisense technology can generally be used to control gene expression through triple-helix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY; 1994). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes. Antisense polynucleotides are generally at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length.

Other agents may modulate transcription by interacting with an metabotropic glutamate receptor gene promoter. Such agents may be identified using standard assays, following isolation of an endogenous metabotropic glutamate receptor gene promoter region. One method for identifying a promoter region uses a PCR-based method to clone unknown genomic DNA sequences adjacent to a known cDNA sequence. This approach may generate a 5' flanking region, which may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection analyses may be used to verify the transcriptional start site deduced from the cDNA.

To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer may be employed. Internal deletion constructs

may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of metabotropic glutamate receptor protein expression In general, the construct with the minimum 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter.

To evaluate the effect of a candidate agent on metabotropic glutamate receptor gene transcription, a promoter or regulatory element thereof may be operatively linked to a reporter gene. Such a construct may be transfected into a suitable host cell, which may be used to screen, for example, a combinatorial small molecule library. Briefly, cells are incubated with the library (*e.g.*, overnight). Cells are then lysed and the supernatant is analyzed for reporter gene activity according to standard protocols. Compounds that result in a decrease in reporter gene activity are inhibitors of metabotropic glutamate receptor gene transcription.

For modulating agents that act directly on a metabotropic glutamate receptor protein, an initial screen to assess the ability of candidate agents to bind to such a protein may be employed, although such binding is not essential for a modulating agent. For identifying agents that bind to a metabotropic glutamate receptor polypeptide, any of a variety of binding assays may be employed, such as standard affinity techniques and yeast two-hybrid screens. In general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μM. An antibody or other modulating agent is said to "specifically bind" to a metabotropic glutamate receptor polypeptide if it reacts at a detectable level with such a polypeptide and does not react detectably with unrelated polypeptides. Such antibody binding properties may be assessed using, for example, an ELISA.

Screens for modulating agents that increase the rate of metabotropic glutamate receptor protein synthesis or stabilize a metabotropic glutamate receptor protein may be readily performed using well known techniques that detect the level of metabotropic glutamate receptor protein or mRNA. Suitable assays include RNA protection assays, *in situ* hybridization, ELISAs, Northern blots and Western blots. Such assays may generally be performed using standard methods (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). For example, to

detect mRNA encoding a metabotropic glutamate receptor protein, a nucleic acid probe complementary to all or a portion of a metabotropic glutamate receptor gene may be employed in a Northern blot analysis of mRNA prepared from suitable cells (*e.g.*, brain, lung, heart, spleen, spinal cord, testis, astrocytes or microglia). To detect a metabotropic glutamate receptor protein, a reagent that binds to the protein (typically an antibody) may be employed within an ELISA or Western assay. Following binding, a reporter group suitable for direct or indirect detection of the reagent is employed (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

To use such assays for identifying a modulating agent, the level of metabotropic glutamate receptor protein or mRNA is evaluated in cells (*e.g.*, transfected HEK 293 cells or Xenopus oocytes) treated with one or more candidate modulating agents. An increase or decrease in metabotropic glutamate receptor protein levels may be measured by evaluating the level of metabotropic glutamate receptor mRNA and/or protein in the presence and absence of candidate modulating agent. In general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μM. A candidate that results in a statistically significant change in the level of metabotropic glutamate receptor mRNA and/or protein is a modulating agent.

Modulating agents that decrease metabotropic glutamate receptor protein levels generally inhibit metabotropic glutamate receptor protein activity. To further evaluate the effect on metabotropic glutamate receptor protein activity, a glutamate binding assay may be performed as described above in the presence and absence of modulating agent. Modulating agents may generally be administered by addition to a cell culture or by the methods described below for *in vivo* administration.

METABOTROPIC GLUTAMATE RECEPTOR POLYPEPTIDE AND MODULATING AGENT MODIFICATIONS AND FORMULATIONS

A metabotropic glutamate receptor polypeptide or modulating agent as described herein may, but need not, be linked to one or more additional molecules. In particular, as discussed below, it may be beneficial for certain applications to link multiple polypeptides and/or modulating agents (which may, but need not, be identical) to a support material, such as a polymeric matrix or a bead or other particle, which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred.

Suitable methods for linking a metabotropic glutamate receptor polypeptide or modulating agent to a support material will depend upon the composition of the support and the intended use, and will be readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may be a direct linkage or may be a linkage by way of a cross-linking agent).

It may be beneficial for certain applications to link a metabotropic glutamate receptor polypeptide or modulating agent to a targeting agent to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a polypeptide or modulating agent enhances the transport of the polypeptide or modulating agent to a target tissue, thereby increasing the local concentration. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Within other embodiments, it may also be possible to target a polynucleotide encoding a polypeptide or modulating agent to a target tissue, thereby increasing the local concentration.

Such targeting may be achieved using well known techniques, including retroviral and adenoviral infection. To treat a patient afflicted with certain conditions, it may be beneficial to deliver a metabotropic glutamate receptor protein polypeptide, polynucleotide or modulating agent to the intracellular space. Such targeting may be achieved using well known techniques, such as through the use of polyethylene glycol or liposomes, as described in Turrens, *Xenobiotica 21*:1033-1040, 1991.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a polypeptide or modulating agent. As used herein, the term "drug" refers to any bioactive agent intended for administration to a mammal to prevent or treat a disease or other undesirable condition.

Within certain aspects of the present invention, one or more polypeptides, polynucleotides or modulating agents as described herein may be present within a pharmaceutical composition or vaccine. A pharmaceutical composition further comprises one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Vaccines may comprise one or more such compounds and a non-specific immune response enhancer. A non-specific immune response enhancer may be any substance that enhances an immune response to an exogenous antigen. Examples of non-specific immune response enhancers include adjuvants and liposomes.

To prepare a pharmaceutical composition, an effective amount of one or more polypeptides, polynucleotides and/or modulating agents is mixed with a suitable pharmaceutical carrier. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application can include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof. In addition, other

pharmaceutically active ingredients and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The number and degree of acceptable side effects depend upon the condition for which the composition is administered. For example, certain toxic and undesirable side effects that are tolerated when treating life-threatening illnesses, such as tumors, would not be tolerated when treating disorders of lesser consequence. The concentration of active component in the composition will depend on absorption, inactivation and excretion rates thereof, the dosage schedule and the amount administered, as well as other factors that may be readily determined by those of skill in the art.

A polypeptide, polynucleotide or modulating agent may be prepared with carriers that protect it against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art. Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polynucleotide, polypeptide or modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Preferably the formulation provides a relatively constant level of modulating agent release. The amount of active component contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). Administration may be effected by incubation of cells *ex vivo* or *in vivo*, such as by topical treatment, delivery by specific carrier or by vascular supply. Appropriate dosages and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient,

the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the polypeptide, polynucleotide and/or modulating agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (*i.e.*, an amount that ameliorates the symptoms or treats or delays or prevents progression of the condition). The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the condition to be alleviated. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art, and for any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

For pharmaceutical compositions comprising polynucleotides, the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, and colloidal dispersion systems such as liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal, as described above). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of

transduced cells) and/or a gene that encodes the ligand for a receptor on a specific target cell (to render the vector target specific).

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.,* an artificial membrane vesicle). RNA, DNA and intact virions can be encapsulated within the aqueous interior and delivered to cells in a biologically active form. The preparation and use of liposomes is well known to those of ordinary skill in the art.

THERAPEUTIC APPLICATIONS

As noted above, metabotropic glutamate receptor polynucleotides, polypeptides and modulating agents may generally be used for the therapy of diseases associated with undesirable levels of glutamate, such as Alzheimer's disease, Parkinson's disease, stroke, depression, anxiety, pain and schizophrenia. Pharmaceutical compositions as provided herein may be administered to a patient, alone or in combination with other therapies, to treat or prevent such diseases.

Modulation of a metabotropic glutamate receptor protein function, either *in vitro* or *in vivo*, may generally be achieved by administering a modulating agent that affects

(inhibits or enhances) metabotropic glutamate receptor protein transcription, translation or activity. The methods described herein may be performed using mammalian cells in culture or within a mammal, such as a human. For treatment of schizophrenia, an increase in glutamate levels is generally desired. In such cases, an agent that inhibits metabotropic glutamate receptor protein function may be administered.

Routes and frequency of administration, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. A suitable dose is an amount of a compound that, when administered as described above, is capable of causing modulation of a activity that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. In general, suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

DIAGNOSTIC APPLICATIONS

In a related aspect of the present invention, kits for detecting metabotropic glutamate receptor proteins are provided. Such kits may be designed for detecting the level of metabotropic glutamate receptor protein or nucleic acid encoding a metabotropic glutamate receptor protein within a sample. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay. A kit for detecting the level of metabotropic glutamate receptor protein or nucleic acid typically contains a reagent that binds to the metabotropic glutamate receptor protein, DNA or RNA. To detect nucleic acid, the reagent may be a nucleic acid probe or a PCR primer. To detect

protein, the reagent is typically an antibody. A kit may also contain a reporter group suitable for direct or indirect detection of the reagent as described above.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Preparation of Novel Metabotropic Glutamate Receptor Protein

This Example illustrates the cloning of cDNA molecules encoding a metabotropic glutamate receptor protein.

The amino acid sequence of the MGLUR5B metabotropic glutamate receptor (*see* Abe et al., *J. Biol. Chem. 267*:13361-13368, 1992; Joly et al., *J. Neurosci. 15*:3970-3981, 1995) was used to query the GenBank EST database using the tBLASTn algorithm. The resulting matches were then scanned to assess their similarity to the native receptor. The nucleotide sequences of those ESTs that were similar, but not identical, to the native receptor were then used to query the non-repetitive GenBank database using the BLASTn algorithm. The EST sequences were used to design oligonucleotide primers for use in RT-PCR reactions to assess the distribution of each metabotropic glutamate receptor gene. Based on this distribution, the tissue with the highest level of expression (brain) was used for both 5' and 3' RACE to allow the isolation of each full length sequence. These products were sequenced to determine the nature of the open reading frames. Following identification of a complete open reading frame for each metabotropic glutamate receptor gene (which includes the presence of at least one in frame translation termination codon upstream of the presumptive start site), primers were designed which were used to amplify the complete open reading frame by PCR. The resulting products were cloned in the mammalian expression vector pcDNA3.1 for transfection into HEK-293 cells.

This process yielded seven new metabotropic glutamate receptor gene sequences (designated GRMX-1a-g, herein). Of these GRMX-1b, GRMX-1c and GRMX-1d encode the same protein. Thus, five new metabotropic glutamate receptor proteins were identified.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various

modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

CLAIMS

1. An isolated polynucleotide that encodes a metabotropic glutamate receptor polypeptide, wherein the polypeptide comprises:

(a) at least 50 consecutive amino acids of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12; or

(b) a variant of any of the foregoing sequences that differs in one or more amino acid substitutions, deletions, additions and/or insertions at no more than 10% of the amino acid residues such that the glutamate-binding activity of the variant is not substantially diminished relative to the metabotropic glutamate receptor protein.

2. A polynucleotide according to claim 1, wherein the polynucleotide comprises at least 60 consecutive nucleotides of sequence recited in any one of SEQ ID NOs:1, 3, 5, 6, 7, 9 or 11.

3. A recombinant expression vector comprising a polynucleotide according to claim 1.

4. A host cell transformed or transfected with an expression vector according to claim 3.

5. An isolated antisense polynucleotide complementary to at least 20 consecutive nucleotides present within a polynucleotide according to claim 1.

6. A recombinant expression vector comprising an antisense polynucleotide according to claim 5.

7. An isolated metabotropic glutamate receptor, comprising:

(a) at least 50 consecutive amino acid residues of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12; or

(b) a variant of any of the foregoing sequences that differs in one or more substitutions, deletions, additions and/or insertions at no more than 10% of amino acid residues, such that the glutamate-binding activity of the variant is not substantially diminished relative to the metabotropic glutamate receptor protein.

8. An isolated metabotropic glutamate receptor according to claim 7 wherein the receptor comprises at least 50 consecutive amino acids of an amino acid sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12.

9. An isolated metabotropic glutamate receptor according to claim 7, wherein the receptor is a homodimer.

10. An isolated metabotropic glutamate receptor according to claim 7, wherein the receptor is a heterodimer.

11. A pharmaceutical composition comprising:

(a) a metabotropic glutamate receptor according to claim 7; and

(b) a physiologically acceptable carrier.

12. A vaccine comprising:

(a) a metabotropic glutamate receptor according to claim 7; and

(b) a non-specific immune response enhancer.

13. An isolated polypeptide, comprising:

(a) at least 50 consecutive amino acid residues of a sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12; or

(b) a variant of any of the foregoing sequences that differs in one or more substitutions, deletions, additions and/or insertions at no more than 10% of amino acid

residues, such that the glutamate-binding activity of the variant is not substantially diminished relative to the metabotropic glutamate receptor protein.

14. An isolated polypeptide according to claim 13, wherein the polypeptide comprises at least 50 consecutive amino acids of an amino acid sequence recited in any one of SEQ ID NOs:2, 4, 8, 10 or 12.

15. A pharmaceutical composition comprising:

(a) a polypeptide according to claim 13; and

(b) a physiologically acceptable carrier.

16. A vaccine comprising:

(a) a polypeptide according to claim 13; and

(b) a non-specific immune response enhancer.

17. A method for preparing a metabotropic glutamate receptor polypeptide, the method comprising:

(a) culturing a host cell transformed or transfected with an expression vector comprising a polynucleotide according to claim 1, wherein the step of culturing is performed under conditions promoting expression of the polynucleotide sequence; and

(b) recovering a metabotropic glutamate receptor polypeptide.

18. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a metabotropic glutamate receptor according to claim 7.

19. A method for screening for an agent that modulates expression of a metabotropic glutamate receptor in a cell, comprising:

(a) contacting a candidate modulator with a cell expressing a metabotropic glutamate receptor polypeptide according to claim 13, wherein the step of contacting is

carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and

(b) subsequently evaluating the effect of the candidate modulator on expression of a metabotropic glutamate receptor mRNA or polypeptide, and therefrom identifying an agent that modulates metabotropic glutamate receptor protein expression in the cell.

20. A method for screening for an agent that modulates a metabotropic glutamate receptor protein activity, comprising:

(a) contacting a candidate modulator with a metabotropic glutamate receptor polypeptide according to claim 13, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and

(b) subsequently evaluating the effect of the candidate modulator on glutamate binding activity of the polypeptide, and therefrom identifying an agent that modulates a metabotropic glutamate receptor protein activity.

21. A method for treating a condition associated with an undesirable level of glutamate in a patient, comprising administering to a patient an agent that decreases expression or activity of a metabotropic glutamate receptor according to claim 7, and thereby treating the condition associated with an undesirable level of glutamate in the patient.

22. A method according to claim 21, wherein the condition is schizophrenia.

23. A method according to claim 21, wherein metabotropic glutamate receptor activity is decreased by inhibiting expression of an endogenous metabotropic glutamate receptor gene.

24. A method according to claim 21, wherein metabotropic glutamate receptor activity is decreased by administering a modulating agent that binds to a metabotropic glutamate receptor protein.

25. A method for modulating metabotropic glutamate receptor activity in a cell, comprising contacting a cell expressing a metabotropic glutamate receptor polypeptide according to claim 13 with an effective amount of an agent that modulates metabotropic glutamate receptor protein activity or expression, and thereby modulating metabotropic glutamate receptor activity in the cell.

Human GRMX-1a DNA sequence

GGCTGTTCTG AGCACGCTGA GCGGAGGGGA TGAGCTTGAG ATCATCTTGG GGGGGAAGCC 60
GGGGACTGGA GAGGCCGGCT CTGCCCTGCT GATCCCCGTG GCCCAACTTT TCGGGGGGCT 120
AGCTAGACCG AGTCTCACTG CTTGCTGCGC AGCCAACAGG GGGGTTTAGA AGATCATGAC 180
CACATGGATC ATCTAACTAA ATGGTACATG GGGACACAAT GGTCCTTTAG AGAATACATC 240
TGAATTGCTG GCTAATTTCT TGATTTGCCA CTCAACGCAG GACATCGCTT GTTCGTAGCT 300
ATCAGAACCC TCCTGAATTT TCCCCACCAT GCTATCTTTA TTAAGCTGGA ACTCCTGTCC 360
TAAAATGGTC CTTCTGTTGA TCCTGTCAGT CTTACTTTTG AAAGAAGATG TCCGTGGGAG 420
TGCACAGTCC AGTGAGAGGA GGGTGGTGGC TCACATGCTG GGTGACATCA TTATTGGAGC 480
TCTCTTTTCT GTTCATCACC AGCCTACTGT GGACGAAGTT CATGAGAGGA AGTGTGGGGC 540
AGTCCGTGAA CAGTATGGCA TTCAGAGAGT GGAGGCCATG CTGCATACCC TGGAAAGGAT 600
CAATTCAGAC CCCACACTCT TGCCCAACAT CACACTGGGC TGTGAGATAA GGGATTCCTG 660
CTGGCATTCG GCTGTGGCCC TAGAGCAGAG CATTGAGTTC ATAAGAGATT CCCTCATTTC 720
TTCGGAAGAG GAAGAGGGCT TGGTGTGCTC TGTGGATGGC TCCTCCTCTT CCTTCCGCTC 780
CAAGAAGCCC ATAGTAGGGG TCATTGGGCC TGGTTCCAGT TCTGTAGCCA TTCAGGTCCA 840
GAATTTGCTC CAGCTTTTCA ACATACCTCA GATTGCTTAC TCAGCAACCA TCATGGATCT 900
GAGTGACAAG ACTCTGTTCA AATATTTCAT GAGGGTTGTG CCTTCAGATG CTCAGCAGGC 960
AAGGTCCATG GTGGACATAG TGAAGAGGTA CAACTGGACC TATGTATCAG CCGTACACAC 1020
AGAAGAGCAG GCTAATTGTG AGGAAGATTG GCTACAAGTG GAGCAGAAAC TGATCTACTA 1080
CAAATGAAAA TGCGCAGTTT TCTATATGAT ACCCAATAAA GTAGATGATA TACTTAAGGA 1140
TATATAAGCA GAAATGCTGA ATGAAAAGGG CATTGTCATG CCAAATAACA TTCATGTCAA 1200
TTGTCATAAG CAGCACAGAA TTTTGAGGTT CAAGCTATTC TCATGCCTCA GCCTCCAGAG 1260
GAGCTAGGCT GGTCTTCAAC TCTTGGCCTC AAGTGATCTG CCTGCTTTGG CCTTCCAAAT 1320
TGCTGGGATT ATGGGCATGA GCCACCACAG CACCCGGCCA GTGCCGCATC TTAGAACACT 1380
CTTGAGGTGC TTTCTGATTT TTAGGCAACT ATGGAGAAAG TGGGATGGAA GCCTTCAAAG 1440
ATATGTCAGC GAAGGAAGGG ATTTGCATCG CCCACTCTTA CAAAATCTAC AGTAATGCAG 1500
GGGAGCAGAG CTTTGATAAG CTGCTGAAGA AGCTCACAAG TCACTTGCCC AAGGCCCGGG 1560
TGGTGGCCTA CTTCTGTGAG GGCATGACGG TGAGAGGTCT GCTGATGGCC ATGAGGCGCC 1620
TGGGTCTAGT GGGAGAATTT CTGCTTCTGG GCACGGAACC AGATGCCATC TTTATTGAGA 1680
TCTCAAAGAA CAGCATCCTA TGGGAAGACA GAAGAAAATG CCAAGGTCGC TTCCTTCAGG 1740
GTTTTGGAGA CATATTACAC AGAAGTGAGT CCGTGCTGCT GCACATGCCC CAGCCTCTGA 1800
ATCTAGAGCT CAGTTCAGGG CCCATCACTG GACTGAGGGA CAGGCTCATC TAATTCTGAG 1860
TGGATATTAC TCTGCATTAT AATGAAGCCA ACAGTCATAT CTTCTGATGT GGAGATTTGA 1920
GAAGCATTTG TATTGGATGT GACCGTCAAA ATGCGCCCCA TATCACTGCA ACACCTACAA 1980
GTTTTCTTGC ATGGGGTGCT CAGACTTTCA CCTCTGGCAA GTATTACTGG GAGGTCCATG 2040
TGGGGGACTC TTGGAATTGG GCTTTCGGTG TTTGTAATAA GTACTGGAAA GGGAAGAATC 2100
AGAATGGCAA TATATATGGA GAGGAGGGAC TCTTTAGTCT TGGGATTGTT AAGAACGACA 2160
TTCAGTGCAG TCTCTTTACC ACCTCCCCAG TTACACTGCA GTATGTCCCA AGACCTACCA 2220
ACCATGTAGG ATTATTCCTG GATTGTGAAG CTAGAACTGT GAGCTTCGTT GATGTTAATC 2280
AAAGCTCCCC TATATACACC ATCCCTAATT GCTCCTTCTC ACCTCCTCTC AGGCCTATCT 2340
TTTGCTGTAT TCATCTCTGA CCAGAGACAA ATCAGAAATG TGTTTATCTG CTGTGGGAAC 2400
CCCTTTATCC CATAAAGCCC TCTTCCTTAT GCCTTATCAA ACAGGACAAA TAGGTTCTGT 2460
TTTATGTCTT GAATTGCATT CTAATGTTAT TAAAACTCAT TTATTGTGTT ACTATTAAAT 2520
GTGGTAAAAA CACTTAAAAA AAAAAAAAAA A 2551

Fig. 1

Human GRMX-Ib DNA sequence

GGCTGTTCTG AGCACGCTGA GCGGAGGGGA TGAGCTTGAG ATCATCTTGG GGGGGAAGCC 60
GGGGACTGGA GAGGCCGGCT CTGCCCTGCT GATCCCCGTG GCCCAACTTT TCGGGGGGCT 120
AGCTAGACCG AGTCTCACTG CTTGCTGCGC AGCCAACAGG GGGGTTTAGA AGATCATGAC 180
CACATGGATC ATCTAACTAA ATGGTACATG GGGACACAAT GGTCCTTTAG AGAATACATC 240
TGAATTGCTG GCTAATTTCT TGATTTGCCA CTCAACGCAG GACATCGCTT GTTCGTAGCT 300
ATCAGAACCC TCCTGAATTT TCCCCACCAT GCTATCTTTA TTAAGCTGGA ACTCCTGTCC 360
TAAAATGGTC CTTCTGTTGA TCCTGTCAGT CTTACTTTTG AAAGAAGATG TCCGTGGGAG 420
TGCACAGTCC AGTGAGAGGA GGGTGGTGGC TCACATGCTG GGTGACATCA TTATTGGAGC 480
TCTCTTTTCT GTTCATCACC AGCCTACTGT GGACGAAGTT CATGAGAGGA AGTGTGGGGC 540
AGTCCGTGAA CAGTATGGCA TTCAGAGAGT GGAGGCCATG CTGCATACCC TGGAAAGGAT 600
CAATTCAGAC CCCACACTCT TGCCCAACAT CACACTGGGC TGTGAGATAA GGGATTCCTG 660
CTGGCATTCG GCTGTGGCCC TAGAGCAGAG CATTGAGTTC ATAAGAGATT CCCTCATTTC 720
TTCGGAAGAG GAAGAGGGCT TGGTGTGCTC TGTGGATGGC TCCTCCTCTT CCTTCCGCTC 780
CAAGAAGCCC ATAGTAGGGG TCATTGGGCC TGGTTCCAGT TCTGTAGCCA TTCAGGTCCA 840
GAATTTGCTC CAGCTTTTCA ACATACCTCA GATTGCTTAC TCAGCAACCA TCATGGATCT 900
GAGTGACAAG ACTCTGTTCA AATATTTCAT GAGGGTTGTG CCTTCAGATG CTCAGCAGGC 960
AAGGTCCATG GTGGACATAG TGAAGAGGTA CAACTGGACC TATGTATCAG CCGTACACAC 1020
AGAAGGTTCA AGCTATTCTC ATGCCTCAGC CTCCAGAGGA GCTAGGCTGG TCTTCAACTC 1080
TTGGCCTCAA GTGATCTGCC TGCTTTGGCC TTCCAAATTG CTGGGATTAT GGGCATGAGC 1140
CACCACAGCA CCCGGCCAGT GCCGCATCTT AGAACACTCT TGAGGTGCTT TCTGATTTTT 1200
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CATGACGGTG AGAGGTCTGC TGATGGCCAT GAGGCGCCTG GGTCTAGTGG GAGAATTTCT 1440
GCTTCTGGGC AGGGAACCAG ATGCCATCTT TATTGAGATC TCAAAGAACA GCATCCTATG 1500
GGAAGACAGA AGAAAATGCC AAGGTCGCTT CCTTCAGGGT TTTGGAGACA TATTACACAG 1560
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TTCCTTATGC CTTATCAAAC AGGACAAATA CGTTCTGTTT TATGTCTTGA ATTGCATTCT 2280
AATGTTATTA AAACTCATTT ATTGTGTTAC TATTAAATGT GGTAAAAACA CTTAAAAAAA 2340
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Fig. 3

Human GRMX-Ia protein sequence

MVLLLILSVLLLKEDVRGSAQSSERRVVAHMLGDIIIGALFSVHHQPTVDEVHERKCGAV
REQYGIQRVEAMLHTLERINSDPTLLPNITLGCEIRDSCWHSAVALEQSIEFIRDSLISS
EEEEGLVCSVDGSSSSFRSKKPIVGVIGPGSSSVAIQVQNLLQLFNIPQIAYSATIMDLS
DKTLFKYFMRVVPSDAQQARSMVDIVKRYNWTYVSAVHTEEQANCEEDWLQVEQKLIYYK

Fig. 2

Human GRMX-Ib,GRMX-Ic and GRMX-Id protein sequence

MVLLLILSVLLLKEDVRGSAQSSERRVVAHMLGDIIIGALFSVHHQPTVDEVHERKCGAV
REQYGIQRVEAMLHTLERINSDPTLLPNITLGCEIRDSCWHSAVALECSIEFIRDSLISS
EEEEGLVCSVDGSSSSFRSKKPIVGVIGPGSSSVAIQVQNLLQLFNIPQIAYSATIMDLS
DKTLFKYFMRVVPSDAQQARSMVDIVKRYNWTYVSAVHTEGSSYSHASASRGARLVFNSW
PQVICLLWPSKLLGLWA

Fig. 4

Human GRMX-Ie protein sequence

MVLLLILSVLLLKEDVRGSAQSSERRVVAHMLGDIIIGALFSVHHQPTVDEVHERKCGAV
REQYGIQRVEAMLHTLERINSDPTLLPNITLGCEIRDSCWHSAVALEQSIEFIRDSLISS
EEEEGLVCSVDGSSSSFRSKKPIVGVIGPGSSSVAIQVQNLLQLFNIPQIAYSATIMDLS
DKTLFKYFMRVVPSDAQQARSMVDIVKRYNWTYVSAVHTEGNYGESGMEAFKDMSAKEGI
CIAHSYKIYSNAGEQSFDKLLKKLTSHLPKARVVAYFCEGMTVRGLLMAMRRLGLVGEFL
LLGRSESVLLHMPQPLNLELSSGPITGLRDRLI

Fig. 8

Human GRMX-If protein sequence

MVLLLILSVLLLKEDVRGSAQSSERRVVAHMLGDIIIGALFSVHHQPTVDEVHERKCGAV
REQYGIQRVEAMLHTLERINSDPTLLPNITLGCEIRDSCWHSAVALEQSIEFIRDSLISS
EEEEGLVCSVDGSSSSFRSKKPIVGVIGPGSSSVAIQVQNLLQLFNIPQIAYSATIMDLS
DKTLFKYFMRVVPSDAQQARSMVDIVKRYNWTYVSAVHTEGNYGESGMEAFKDMSAKEGI
CIAHSYKIYSNAGEQSFDKLLKKLTSHLPKARVVAYFCEGMTVRGLLMAMRRLGLVGEFL
LLGREPDAIFIEISKNSILWEDRRKCQGRFLQK

Fig. 10

Human GRMX-Ic DNA sequence

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CACATGGATC ATCTAACTAA ATGGTACATG GGGACACAAT GGTCCTTTAG AGAATACATC 240
TGAATTGCTG GCTAATTTCT TGATTTGCCA CTCAACGCAG GACATCGCTT GTTCGTAGCT 300
ATCAGAACCC TCCTGAATTT TCCCCACCAT GCTATCTTTA TTAAGCTGGA ACTCCTGTCC 360
TAAAATGGTC CTTCTGTTGA TCCTGTCAGT CTTACTTTTG AAAGAAGATG TCCGTGGGAG 420
TGCACAGTCC AGTGAGAGGA GGGTGGTGGC TCACATGCTG GCTGACATCA TTATTGGAGC 480
TCTCTTTTCT GTTCATCACC AGCCTACTGT GGACGAAGTT CATGAGAGGA AGTGTGGGGC 540
AGTCCGTGAA CAGTATGGCA TTCAGAGAGT GGAGGCCATG CTGCATACCC TGGAAAGGAT 600
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CATGACGGTG AGAGGTCTGC TGATGGCCAT GAGGCGCCTG GGTCTAGTGG GAGAATTTCT 1440
GCTTCTGGGC AGGGAACCAG ATGCCATCTT TATTGAGATC TCAAAGAACA GCATCCTATG 1500
GGAAGACAGA AGAAAATGCC AAGGTCGCTT CCTTCAGAAG TGAGTCCGTG CTGCTGCACA 1560
TGCCCCAGCC TCTGAATCTA GAGCTCAGTT CAGGGCCCAT CACTGGACTG AGGGACAGGC 1620
TCATCTAATT CTGAGTGGAT ATTACTCTGC ATTATAATGA AGCCAACAGT CATATCTTCT 1680
GATGTGGAGA TTTGAGAAGC ATTTGTATTG GATGTGACCG TCAAAATGCG CCCCATATCA 1740
CTGCAACACC TACAAGTTTT CTTGCATGGG GTGCTCAGAC TTTCACCTCT GGCAAGTATT 1800
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CTCTCAGGCC TATCTTTTGC TGTATTCATC TCTGACCAGA GACAAATCAG AAATGTGTTT 2160
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GTGTTACTAT TAAATGTGGT AAAAACACTT AAAAAAAAAA AAAAAA 2326
```

Fig. 5

Human GRMX-Id DNA sequence

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TGCACAGTCC AGTGAGAGGA GGGTGGTGGC TCACATGCTG GGTGACATCA TTATTGGAGC 480
TCTCTTTTCT GTTCATCACC AGCCTACTGT GGACGAAGTT CATGAGAGGA AGTGTGGGGC 540
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CAATTCAGAC CCCACACTCT TGCCCAACAT CACACTGGGC TGTGAGATAA GGGATTCCTG 660
CTGGCATTCG GCTGTGGCCC TAGAGCAGAG CATTGAGTTC ATAAGAGATT CCCTCATTTC 720
TTCGGAAGAG GAAGAGGGCT TGGTGTGCTC TGTGGATGGC TCCTCCTCTT CCTTCCGCTC 780
CAAGAAGCCC ATAGTAGGGG TCATTGGGCC TGGTTCCAGT TCTGTAGCCA TTCAGGTCCA 840
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AGAAGGTTCA AGCTATTCTC ATGCCTCAGC CTCCAGAGGA GCTAGGCTGG TCTTCAACTC 1080
TTGGCCTCAA GTGATCTGCC TGCTTTGGCC TTCCAAATTG CTGGGATTAT GGGCATGAGC 1140
CACCACAGCA CCCGGCCAGT GCCGCATCTT AGAACACTCT TGAGGTGCTT TCTGATTTTT 1200
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GCTGAAGAAG CTCACAAGTC ACTTGCCCAA GGCCCGGGTG GTGGCCTACT TCTGTGAGGG 1380
CATGACGGTG AGAGGTCTGC TGATGGCCAT GAGGCGCCTG GGTCTAGTGG GAGAATTTCT 1440
GCTTCTGGGC AGAAGTGAGT CCGTGCTGCT GCACATGCCC CAGCCTCTGA ATCTAGAGCT 1500
CAGTTCAGGG CCCATCACTG GACTGAGGGA CAGGCTCATC TAATTCTGAG TGGATATTAC 1560
TCTGCATTAT AATGAAGCCA ACAGTCATAT CTTCTGATGT GGAGATTTGA GAAGCATTTG 1620
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TATATACACC ATCCCTAATT GCTCCTTCTC ACCTCCTCTC AGGCCTATCT TTTGCTGTAT 2040
TCATCTCTGA CCAGAGACAA ATCAGAAATG TGTTTATCTG CTGTGGGAAC CCCTTTATCC 2100
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CACTTAAAAA AAAAAAAAAA A                                          2241
```

Fig. 6

Human GRMX-Ie DNA sequence

GGCTGTTCTG AGCACGCTGA GCGCAGGGGA TGAGCTTGAG ATCATCTTGG GGGGCAAGCC 60
GGGGACTGGA GAGGCCGGCT CTGCCCTGCT GATCCCCGTG GCCCAACTTT TCGGGGGGCT 120
AGCTAGACCG AGTCTCACTG CTTGCTGCGC AGCCAACAGG GGGGTTTAGA AGATCATGAC 180
CACATGGATC ATCTAACTAA ATGGTACATG GGGACACAAT GGTCCTTTAG AGAATACATC 240
TGAATTGCTG GCTAATTTCT TGATTTGCCA CTCAACGCAG GACATCGCTT GTTCGTAGCT 300
ATCAGAACCC TCCTGAATTT TCCCCACCAT GCTATCTTTA TTAAGCTGGA ACTCCTGTCC 360
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TGCACAGTCC AGTGAGAGGA GGGTGGTGGC TCACATGCTG GGTGACATCA TTATTGGAGC 480
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TTCGGAAGAG GAAGAGGGCT TGGTGTGCTC TGTGGATGGC TCCTCCTCTT CCTTCCGCTC 780
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GAGTGACAAG ACTCTGTTCA AATATTTCAT GAGGGTTGTG CCTTCAGATG CTCAGCAGGC 960
AAGGTCCATG GTGGACATAG TGAAGAGGTA CAACTGGACC TATGTATCAG CCGTACACAC 1020
AGAAGGCAAC TATGGAGAAA GTGGGATGGA AGCCTTCAAA GATATGTCAG CGAAGGAAGG 1080
GATTTGCATC GCCCACTCTT ACAAAATCTA CAGTAATGCA GGGGAGCAGA GCTTTGATAA 1140
GCTGCTGAAG AAGCTCACAA GTCACTTGCC CAAGGCCCGG GTGGTGGCCT ACTTCTGTGA 1200
GGGCATGACG GTGAGAGGTC TGCTGATGGC CATGAGGCGC CTGGGTCTAG TGGGAGAATT 1260
TCTGCTTCTG GGCAGGGAAC CAGATGCCAT CTTTATTGAG ATCTCAAAGA ACAGCATCCT 1320
ATGGGAAGAC AGAAGAAAAT GCCAAGGTCG CTTCCTTCAG GGTTTTGGAG ACATATTACA 1380
CAGAAGTGAG TCCGTGCTGC TGCACATGCC CCAGCCTCTG AATCTAGAGC TCAGTTCAGG 1440
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AGAGGAGGGA CTCTTTAGTC TTGGGATTGT TAAGAACGAC ATTCAGTGCA GTCTCTTTAC 1800
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TCTAATGTTA TTAAAACTCA TTTATTGTGT TACTATTAAA TGTGGTAAAA ACACTTAAAA 2160
AAAAAAAAA AA 2172

Fig. 7

Human GRMX-If DNA sequence

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Fig. 9

Human GRMX-Ig DNA sequence

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Fig. 11

Human GRMX-Ig protein sequence

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Fig. 12

SEQUENCE LISTING

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Schwarz, David A.
Maki, Richard A.

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Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg
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Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn
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Val Asp Glu Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr
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Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn
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Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg
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Asp Ser Cys Trp His Ser Ala Val Ala Leu Glu Gln Ser Ile Glu Phe
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Ile Arg Asp Ser Leu Ile Ser Ser Glu Glu Glu Glu Gly Leu Val Cys
115 120 125

Ser Val Asp Gly Ser Ser Ser Ser Phe Arg Ser Lys Lys Pro Ile Val
130 135 140

Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn
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Leu Leu Gln Leu Phe Asn Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ile
165 170 175

Met Asp Leu Ser Asp Lys Thr Leu Phe Lys Tyr Phe Met Arg Val Val
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Pro Ser Asp Ala Gln Gln Ala Arg Ser Met Val Asp Ile Val Lys Arg
195 200 205

Tyr Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Ser Ser Tyr
210 215 220

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Val Asp Glu Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr
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Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn
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Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg

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INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
PCT/US 00/17798

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N5/10 C07K14/705 C07K16/28 A61K38/17
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MINAKAMI R. ET AL.: "Molecular cloning and the functional expression of two isoforms of human metabotropic glutamate receptor subtype 5" BIOCHEM. BIOPHYS. RES. COM., vol. 199, no. 3, 1994, pages 1136-1143, XP002149696 the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	26/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/17798

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
X	ABE T ET AL: "MOLECULAR CHARACTERIZATION OF A NOVEL METABOTROPIC GLUTAMATE RECEPTOR MGLUR5 COUPLED TO INOSITOL PHOSPHATE/CA2+ SIGNAL TRANSDUCTION" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 267, no. 19, 5 July 1992 (1992-07-05), pages 13361-13368, XP000605387 ISSN: 0021-9258 cited in the application the whole document	1-20
P,X	WO 99 38975 A (RIKKE BRAD A ;SIKELA JAMES M (US); JOHNSON THOMAS E (US); SIMPSON) 5 August 1999 (1999-08-05) abstract sequences claims 1-38	1-20

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 00 17798

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25, partly

An isolated polynucleotide that encodes a metabotropic glutamate receptor polypeptide, wherein the polypeptide comprises a least 50 consecutive amino acids of seq. ID 2, or a variant or homolog thereof.

Corresponding polypeptides, antisense, vectors, recombinant host cells, antibodies, therapeutic and screening uses.

2. Claims: 1-25, partly

Idem as subject-matter 1, but limited to seq. ID 4.

3. Claims: 1-25, partly

Idem as subject-matter 1, but limited to seq. ID 8.

4. Claims: 1-25, partly

Idem as subject-matter 1, but limited to seq. ID 10.

5. Claims: 1-25, partly

Idem as subject-matter 1, but limited to seq. ID 12.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 00/17798

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 21-25

Claims 21-25 refer to agonists and antagonists (modulators) of the metabotrobic glutamate receptors claimed, without however giving a true technical characterization. Moreover, no such compounds are sufficiently described or defined in the application in compliance with Art. 5 and 6 PCT. No search can be performed for such purely speculative claims, the wording of which is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ional Application No
PCT/US 00/17798

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9938975 A	05-08-1999	AU 2486999 A	16-08-1999